

CONTROL OF *LISTERIA MONOCYTOGENES* ON FRANKFURTERS WITH ANTIMICROBIALS AND HYDRODYNAMIC PRESSURE PROCESSING*

J.R. PATEL¹, G.C. SANGLAY and M.B. SOLOMON

*Food Technology and Safety Laboratory
Beltsville Agricultural Research Center
Agriculture Research Service, USDA
Beltsville, MD 20705-2350*

Accepted for Publication October 1, 2007

ABSTRACT

*The antilisterial activity of sodium diacetate and pediocin (ALTA 2341) on frankfurters was evaluated in combination with hydrodynamic pressure processing (HDP). Minimum inhibitory concentration (MIC) of these strains as determined in tryptic soy broth was 0.4% and >600 AU/mL, for sodium diacetate and ALTA 2341, respectively. Frankfurters were surface inoculated with a five-strain mixture of *Listeria monocytogenes* after dipping in (1) control (sterile water); (2) 5% sodium diacetate; (3) 3% ALTA 2341 (P1); (4) 6% ALTA 2341 (P2); (5) P1 + sodium diacetate; and (6) P2 + sodium diacetate for 5 min. The frankfurters were vacuum-packaged and treated with hydrodynamic pressure or without HDP. Frankfurters were analyzed at 0, 7, 14 and 28 d for pH, *L. monocytogenes* (MOX) and aerobic (TSAYE) cell populations during storage at 4°C. Antimicrobials and HDP treatment did not change ($P < 0.05$) the frankfurter pH. HDP treatment significantly reduced ($1 \log_{10}$ cfu/g) *L. monocytogenes* populations in frankfurters. Dipping treatments containing sodium diacetate or ALTA 2341 did not significantly reduce *L. monocytogenes* in frankfurters. There was a no synergistic effect between ALTA 2341 and HDP for inhibition of *L. monocytogenes*. The reduction pattern in aerobic cell populations during storage of antimicrobials and HDP-treated frankfurters was similar to that of *L. monocytogenes* reduction. These results indicate that the ALTA 2341 preparation was not efficient enough to kill *L. monocytogenes*. Other antimicrobials should be evaluated in conjunction with HDP treatment for synergistic inhibition of *L. monocytogenes* during storage at 4°C.*

* The United States Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product and the use of the name by USDA implies no approval of the product to the exclusion of other products that may also be available.

¹ Corresponding author. TEL: 301-504-7003; FAX: 301-504-8438; EMAIL: jitu.patel@ars.usda.gov

PRACTICAL APPLICATIONS

Since the early 1980s, when *Listeria monocytogenes* was first recognized as an emerging food-borne pathogen, numbers of outbreaks of human listeriosis have been reported. Many of these outbreaks are associated with processed meats. Because of the high mortality rate associated with listeriosis, a zero tolerance approach for *L. monocytogenes* in ready-to-eat meats and poultry products has been selected by many countries, including the U.S.A. We reported significant *L. monocytogenes* reduction in frankfurters using hydrodynamic pressure (HDP) treatment. HDP processing using a nonexplosive energy source can be used to reduce *L. monocytogenes* in frankfurters. While pediocin and organic acids used in our study reduce *L. monocytogenes* during storage, their practical use is not significant as the reduction is marginal. Other antimicrobials should be evaluated in conjunction with high-pressure processing for required reduction of *L. monocytogenes* during storage at 4°C.

INTRODUCTION

Listeria monocytogenes has been recognized as a significant pathogen for various foods, but most especially ready-to-eat (RTE) meat products. Of the 76 million estimated cases of foodborne illness in the U.S.A. each year, *L. monocytogenes* is responsible for approximately 2,518 cases of illness and 504 deaths (Mead *et al.* 1999). Listeriosis is known to be potentially fatal to certain population groups such as the elderly or the immunocompromised, but is of particular concern to pregnant mothers and newborns. *L. monocytogenes* is notoriously difficult to completely remove from the processing environment (Samelis and Metaxopoulos 1999) and can contaminate food products by either being present in the raw ingredients, or when post-thermally treated products come into contact with biofilms or improperly cleaned/sanitized equipment (United States Department of Agriculture [USDA] Food Safety and Inspection Service 2003). In the U.S.A., there is a zero-tolerance policy on *L. monocytogenes* in RTE meat and poultry products. RTE products are considered adulterated if *L. monocytogenes* is found in 25 g samples tested (Jay 1996). Some notable outbreaks of *L. monocytogenes* include a multistate outbreak in 2002 involving sliceable turkey deli meat resulting in 46 confirmed cases, seven deaths and three miscarriages (Centers for Disease Control and Prevention [CDC] 2002). Another outbreak in 1998 involved beef franks which resulted in 15 deaths (CDC 1998).

To prevent *L. monocytogenes* contamination of food products, researchers have examined antimicrobials and alternative processing technologies to

reduce or eliminate the presence of this pathogen. The antimicrobial properties of organic acids such as sodium lactate (SL) and sodium diacetate have been reported. Uhart *et al.* (2004) demonstrated that 3% SL and 6% sodium diacetate, alone or in combination, produced a 1–2 log reduction of *L. monocytogenes* on beef franks after 3 weeks of storage at 4C. Barmpalia *et al.* (2004) found that pork frankfurters formulated with a combination of 1.8% SL and 0.25% sodium diacetate produced complete inhibition of *L. monocytogenes* during 40 d of storage at 10C, while formulated franks dipped in lactic and acetic acid solutions produced a 0.6–1.0 log cfu/cm² over 28–40 d. Lu *et al.* (2005) found that frankfurters dipped in 6% sodium diacetate were more effective in reducing *L. monocytogenes* populations than treatments using SL, sodium diacetate and potassium benzoate (PB), and SL + sodium diacetate + PB.

Bacteriocins, such as nisin and pediocin, have also been identified to demonstrate antilisterial activity. These antimicrobials are known to be effective against gram-positive bacteria, but ineffective against gram-negative bacteria unless a chelator, such as ethylene diamino tetra acetate, is used (Gill and Holley 2000). In commercially prepared franks formulated with potassium lactate and sodium diacetate, franks packaged with nisin-coated casings produced a 1.15 log cfu reduction of *L. monocytogenes* after 90 d of storage, as compared to a 0.95 log cfu reduction in franks that did not use the nisin-coated casings (Luchansky and Call 2004). Beef franks dipped in a pediocin (6,000 AU) solution produced a less than 1 log reduction of a four-strain cocktail of *L. monocytogenes* after 2 to 3 weeks of storage at 4C (Uhart *et al.* 2004).

Research has been conducted to determine the effectiveness of the hydrodynamic pressure process (HDP) to reduce or eliminate microorganisms from food products. The process was originally developed to enhance meat tenderness. HDP utilizes a high-energy explosive, detonated in a liquid medium, to generate supersonic shockwaves that can disrupt muscle fibers in meat products (Solomon *et al.* 1997). Research has demonstrated the ability of HDP to reduce indigenous bacterial populations on beef and pork stew pieces, ground beef and intact beef muscle (Williams-Campbell and Solomon 2000, 2002; Schilling *et al.* 2003). HDP has also demonstrated reductions of *Salmonella* populations in minced chicken (Patel *et al.* 2006) and *L. monocytogenes* populations on beef surfaces (Patel and Solomon 2005). Research has also found that coupling nisin with HDP produced a greater than 2 log reduction of *L. monocytogenes* populations on beef franks after 28 d of refrigerated storage (Patel *et al.* 2007).

The objective of this study was to investigate the ability of HDP, in conjunction with varying concentrations of sodium diacetate and pediocin (ALTA 2341) alone or in combination, to reduce populations of *L. monocytogenes* populations on beef franks after 28 d of storage at 4C.

MATERIALS AND METHODS

Bacterial Strains

A five-strain cocktail of *L. monocytogenes* was used in this study. Strain Scott A was obtained from culture collection at our laboratory. Four meat isolates were obtained from Dr. Sadhana Ravishankar (National Center for Food Safety and Technology, Summit-Argo, IL): 101 M (serotype 4b, beef and pork sausage), 108 M (serotype 1/2b, hard salami), H7776 (serotype 4b, frankfurter) and F6854 (serotype 1/2a, frankfurter). All strains were maintained in sterile 2.0 mL cryovials (Nalgene, Rochester, NY) containing tryptic soy broth supplemented with 0.6% yeast extract (TSBYE, Difco Laboratories, Detroit, MI) and 20% glycerol (Fisher Scientific, Suwanee, GA). Cryovials were stored at -80°C until ready for use. Individual cultures were thawed at room temperature for 10–15 min and were transferred by single loopful to 10 mL TSBYE tubes and incubated at 35°C for 24 h. After two successive transfers, all five strains were mixed in equal proportions in a sterile test tube and vortexed to prepare the cocktail.

Antimicrobials and Frankfurters

Commercially prepared beef frankfurters were purchased from local supermarkets. Two antimicrobials were used in this study. Sodium diacetate was obtained from Sigma-Aldrich (Allentown, PA). Pediocin (P), designated as ALTA 2341, was obtained from Quest International, Inc. (Sarasota, FL).

Minimum Inhibitory Concentration (MIC) Assay

Uhart *et al.* (2004) describe the procedure for determining the MIC of each of the antimicrobials on individual *L. monocytogenes* strains. For sodium diacetate, solutions of varying concentrations (0 to 0.8%) were prepared in 10-mL aliquots of TSBYE. Each solution was inoculated individually with 0.1 mL ($\sim 7 \log_{10}$ cfu/mL) of each *L. monocytogenes* strain. Tubes were incubated at 35°C for 24 h. The MIC was recorded as the lowest concentration that resulted in no visible turbidity after incubation. Pediocin (ALTA 2341) solutions were prepared by dissolving the pediocin in sterile deionized water. Tryptic soy agar plates with yeast extract (TSAYE; Difco) were overlaid with 5 mL semisolid TSAYE (0.8% agar) inoculated with $\sim 8 \log_{10}$ cfu/mL of each individual *L. monocytogenes* strain and allowed to solidify for 30 min. The pediocin solution was serially diluted with sterile deionized water. Each dilution was spot-inoculated (five spots of 5 μL) onto each plate. Plates were incubated at 35°C for 24 h. MIC was recorded as the lowest dilution that produced a definite zone of inhibition on the bacterial lawns.

Sample Preparation

Thirty-six beef franks were divided into two groups for no-HDP and HDP treatment. There were six treatments which consisted of the following: (1) sterile deionized water (control); (2) 3% sodium diacetate; (3) 3% pediocin (P1); (4) 6% pediocin (P2); (5) sodium diacetate + P1; and (6) sodium diacetate + P2. Treatment solutions were prepared in sterilized ninth-size polycarbonate food pans (Ace Mart Restaurant Supply, San Antonio, TX). Beef franks were dipped into treatment solutions for 5 min at room temperature (25C) and then allowed to air-dry on sterile racks for 10 min. Following drying, the franks were then dipped into sterilized sixth-size stainless steel steam table pans (Ace Mart Restaurant Supply) containing $7 \log_{10}$ cfu/mL of the *L. monocytogenes* cocktail for 5 min at 25C to allow bacterial attachment on the surface of the franks. The franks were then allowed to air-dry on sterile racks for 10 min.

Beef franks were separated by their designated treatments and placed into 15.2×25.4 cm ($6 \times 10''$) nylon vacuum pouches (3 mL standard barrier; Cryovac Sealed Air Corporation, Duncan, SC) and vacuum-packaged (Model LV 10G, Hollymatic Corporation, Countryside, IL). Beef frank packages for HDP treatment were further packaged in 35.5×66.0 cm Boneguard bags (Cryovac Sealed Air) and heat shrunk 88C water for 1 s to remove air pockets. Heat-shrinking step did not kill *L. monocytogenes* in beef franks. Following HDP treatment, packages were stored at 4C for up to 28 d.

HDP

HDP was conducted indoors in a 54-L stainless steel cylindrical shock-wave container. Boneguard bags containing the beef franks were secured to the bottom of the container and the container was filled with water and ice to reach a temperature of 4C. A 100-g binary explosive (rectangle-shaped) was suspended in the water 30.5 cm above the package surface. The steel container lid was secured and locked, and the explosive was detonated.

Microbiological Analyses

Following treatment, packages were aseptically opened and the beef franks were placed on sterile trays. The rounded edges of the franks were removed using sterile knives in order to obtain uniform sample size. An approximately 25 g (~ 2.54 cm) sample was removed and placed into a sterile stomacher bag with a filter (Microbiology International, Frederick, MD). Samples were serially diluted with sterile 0.1% peptone water (Difco) using a Dilumat 3 automatic diluter (Microbiology International). The sample was pummeled in a BagMixer (Interscience, St. Nom, France) for 2 min. Samples

were further diluted in sterile 99 mL 0.1% peptone water (Difco) and spiral plated (100 μ L; WASP2, Microbiology International) onto duplicate TSAYE and modified Oxford agar (MOX, BD, Sparks, MD) plates. Plates were incubated at 35C for 48 h and then counted using the ProtoCOL automated colony counter (Microbiology International).

pH Measurement

Peachey *et al.* (2002) described the procedure for measuring the pH of meat samples. Briefly, a 2–2.5-g sample was placed into a sterile 50-mL conical centrifuge tube (VWR International, West Chester, PA) containing 10 mL of iodoacetate solution (5 mM iodoacetate in 150 mM KCl, Sigma-Aldrich). The sample was homogenized using a Polytron Homogenizer with a 12-mm foam-reducing generator (Brinkmann Instruments, Westbury, NY). The pH was measured using a Denver Instrument Model 250 pH meter (Denver, CO).

Statistical Analyses

The mean values of two plates from each of three replicates were converted to log₁₀ cfu/g. Analysis of variance was performed using a “Proc Mixed” statement (SAS 8.2, SAS Institute, Inc., Cary, NC) for effects of treatment, antimicrobials, storage time and interaction. The Sidak adjustment was utilized to protect against inflation of the Type 1 error. In all cases, a statistical significance level of $P < 0.05$ was used.

RESULTS

MIC Assay

The MICs for sodium diacetate were 0.4% for all strains. Inhibitory activity of ALTA 2341 varied with the different strains of *Listeria*. The MIC for ALTA 2341 were 600 AU/mL (3%) for Scott A, 800 AU/mL for 108 M and 1,000 AU/mL for strains 101 M. The MIC for strains H7776 and F6854 could not be reached as these two strains showed weak growth at 1,200 AU/mL (6% ALTA 2341).

Effect of Antimicrobials on pH

The initial pH of the frankfurter was 6.02 (Table 1). ALTA 2341 dipping treatment at 3 or 6% did not influence the pH. Sodium diacetate alone or in combination with ALTA 2341 reduced the pH to 5.75; however, the reduction was not significant. There was no effect of HDP treatment on pH of frankfurt-

TABLE 1.
LEAST SQUARE MEANS FOR PH VALUES OF FRANKFURTERS TREATED WITH
ANTIMICROBIALS FOLLOWED BY HDP AND STORED AT 4C

Treatment*	Dip solution†	Time (days)			
		0	7	14	28
Control	Water	6.02 (0.02)‡	5.99 (0.14)	6.00 (0.12)	6.08 (0.05)
	SDA	5.75 (0.04)	5.87 (0.03)	5.89 (0.06)	5.90 (0.06)
	P1	5.97 (0.04)	5.96 (0.07)	6.00 (0.06)	5.99 (0.08)
	P2	5.98 (0.03)	5.96 (0.09)	5.98 (0.03)	5.99 (0.07)
	SDA + P1	5.75 (0.06)	5.84 (0.02)	5.83 (0.08)	5.84 (0.07)
	SDA + P2	5.75 (0.05)	5.84 (0.03)	5.88 (0.02)	5.85 (0.07)
HDP	Water	6.00 (0.02)	6.00 (0.06)	6.02 (0.06)	6.05 (0.04)
	SDA	5.79 (0.06)	5.88 (0.03)	5.87 (0.07)	5.89 (0.10)
	P1	6.00 (0.04)	5.99 (0.06)	5.96 (0.10)	5.97 (0.12)
	P2	5.99 (0.06)	5.96 (0.06)	5.95 (0.07)	5.89 (0.10)
	SDA + P1	5.81 (0.06)	5.88 (0.04)	5.87 (0.08)	5.85 (0.10)
	SDA + P2	5.80 (0.05)	5.87 (0.02)	5.89 (0.04)	5.85 (0.07)

* Control, not treated with HDP.

† Water, control; SDA, sodium diacetate; P1, 3% pediocin; P2, 6% pediocin.

‡ Values in parentheses are standard deviations.

HDP, hydrodynamic pressure processing.

ers. A modest pH increase of up to 0.1 unit was noticed in both antimicrobial and HDP-treated samples during refrigerated storage, except in HDP and 6% ALTA 2341-treated frankfurters where the pH reduced by 0.1 unit.

L. monocytogenes Inhibition by Antimicrobials

No *L. monocytogenes* were detected on MOX plates and very few (<1 log₁₀ cfu/g) aerobic populations were detected on TSAYE plates from uninoculated and untreated beef frankfurters. The behavior of *L. monocytogenes* on frankfurters after treatment with antimicrobials and HDP is presented in Fig. 1A. Initial *L. monocytogenes* populations in untreated beef franks (inoculated controls) was 5.12 log₁₀ cfu/g. The HDP treatment significantly reduced *L. monocytogenes* populations to 4.30 log₁₀ cfu/g. Dipping in ALTA 2341, sodium diacetate or their combinations did not reduce *L. monocytogenes* populations in frankfurters. *L. monocytogenes* populations on beef franks treated with antimicrobials (sodium diacetate, ALTA 2341 or their combinations) and HDP were not significantly reduced when compared to counts on beef franks that were only treated with HDP. The combination of dipping in sodium diacetate and ALTA 2341 (6%), and HDP treatment reduced *L. monocytogenes* populations to 4.0 log₁₀ cfu/g from initial populations of 5.12 log₁₀ cfu/g.

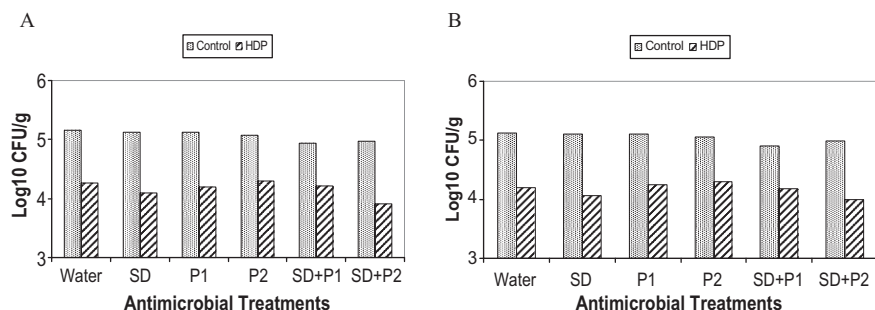


FIG. 1. SURVIVAL OF *LISTERIA MONOCYTOGENES* AND AEROBIC MICROORGANISMS ON THE SURFACE OF FRANKFURTERS TREATED WITH ANTIMICROBIALS AND POSTPACKAGING HYDRODYNAMIC PRESSURE TREATMENT (A) AEROBIC PLATE COUNTS ON TSAYE AGAR AND (B) *L. MONOCYTOGENES* COUNTS ON MOX AGAR (□ CONTROL, NONTREATED WITH HYDRODYNAMIC PRESSURE, ▨ HDP, HYDRODYNAMIC PRESSURE TREATMENT; SD, SODIUM DIACETATE; P1, 3% PEDIOCIN; P2, 6% PEDIOCIN; SD + P1, SODIUM DIACETATE AND 3% PEDIOCIN; SD + P2, SODIUM DIACETATE AND 6% PEDIOCIN CONCENTRATIONS WERE USED FOR DIPPING) (STANDARD DEVIATION VARIED FROM 0.05 TO 0.20)

L. monocytogenes populations recovered on TSAYE were identical to those recovered on MOX agar (Fig. 1B). Bacterial populations recovered on TSAYE were identical or greater than those recovered on MOX agar. HDP treatment significantly reduced aerobic populations on frankfurters to 4.27 log₁₀ cfu/g from 5.16 log₁₀ cfu/g. Colonies grown on TSAYE were predominantly *L. monocytogenes* resembling uniform appearance of typical *Listeria* colonies. The effect of antimicrobials (sodium diacetate, ALTA 2341 and their combination) and HDP followed a similar pattern of bacterial recovery on TSAYE medium as observed on MOX medium.

Effect of Antimicrobials during Storage

During the 28-d storage period, *L. monocytogenes* counts were reduced in frankfurters treated with antimicrobials and their combinations (Fig. 2A). *L. monocytogenes* populations detected following 7-d storage of antimicrobial-treated frankfurters were significantly lower than initial *L. monocytogenes* populations (day 0) from correspondingly antimicrobial-treated frankfurters, with an exception of the sodium diacetate + 3% ALTA 2341 treatment. The *L. monocytogenes* populations recovered on 28-d stored frankfurters that were treated with 3% ALTA 2341 (3.98 log₁₀ cfu/g), 6% ALTA 2341 (3.89 log₁₀ cfu/g), sodium diacetate (4.07 log₁₀ cfu/g), sodium diacetate + 3% ALTA 2341 (3.81 log₁₀ cfu/g) or sodium diacetate + 6% ALTA 2341 (3.88 log₁₀ cfu/g) were lower ($P > 0.05$) compared to the *L.*

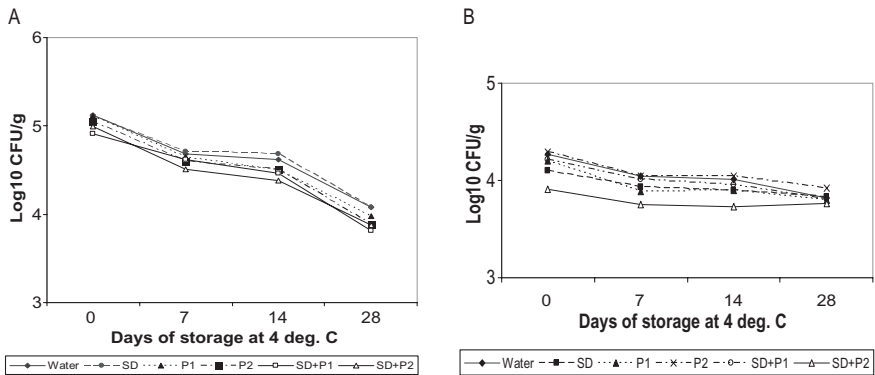


FIG. 2. SURVIVAL AND GROWTH OF *LISTERIA MONOCYTOGENES* AND AEROBIC BACTERIA ON THE SURFACE OF FRANKFURTERS TREATED WITH ANTIMICROBIALS DURING STORAGE AT 4C (A) *L. MONOCYTOGENES* ON MOX AGAR AND (B) AEROBIC PLATE COUNTS ON TSAYE AGAR (SD, SODIUM DIACETATE, P1, 3% PEDIOCIN; P2, 6% PEDIOCIN; SD + P1, SODIUM DIACETATE AND 3% PEDIOCIN; SD + P2, SODIUM DIACETATE AND 6% PEDIOCIN CONCENTRATIONS WERE USED FOR DIPPING) (STANDARD DEVIATION VARIED FROM 0.05 TO 0.65)

monocytogenes populations obtained from their corresponding day 0 samples. The reduction in *L. monocytogenes* populations on treated frankfurters obtained on specific sampling days 7, 14 or 28 was not significantly different. Aerobic bacterial populations in antimicrobial-treated frankfurters decreased during 4C storage (Fig. 2B). Cell populations recovered on TSAYE were identical to those recovered on MOX agar. *L. monocytogenes* populations were significantly reduced in all but sodium diacetate + 3% ALTA 2341 antimicrobial-treated frankfurters following 7 d of storage. When compared at each sampling period of 7, 14 or 28 d, aerobic cell populations obtained from all antimicrobial-treated frankfurters were similar ($P > 0.05$).

A trend of *L. monocytogenes* reduction on beef franks treated with antimicrobials and HDP was similar to that on beef franks treated with only antimicrobials (Fig. 3a). Marginal *L. monocytogenes* reduction (ca. 0.5 log₁₀ cfu/g) was observed during storage of antimicrobials- and HDP-treated frankfurters. During storage of 7, 14 or 28 d, *L. monocytogenes* populations detected in frankfurters that were treated with antimicrobials and HDP were not significantly different from their initial *L. monocytogenes* populations (day 0), except the 3% ALTA 2341 and HDP treatment combination. There was no synergistic antilisterial effect of HDP and antimicrobials. Combining sodium diacetate with 3% ALTA 2341 prior to the HDP treatment did not exert additional *L. monocytogenes* reduction on frankfurters as evident from

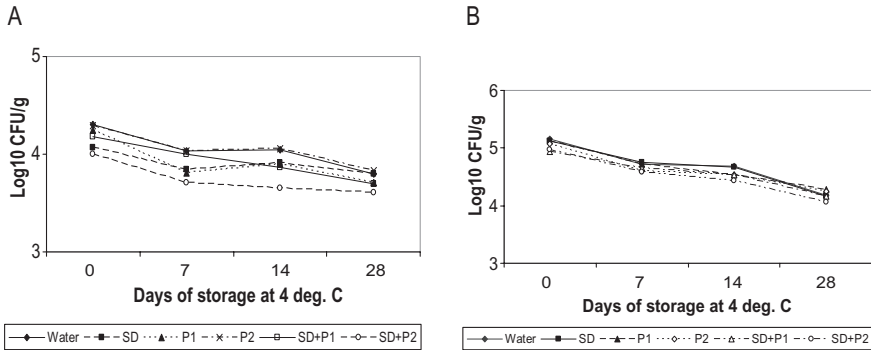


FIG. 3. SURVIVAL AND GROWTH OF *LISTERIA MONOCYTOGENES* AND AEROBIC BACTERIA ON THE SURFACE OF FRANKFURTERS TREATED WITH ANTIMICROBIALS AND HYDRODYNAMIC PRESSURE PROCESSING DURING STORAGE AT 4C (A) *L. MONOCYTOGENES* ON MOX AGAR AND (B) AEROBIC PLATE COUNTS ON TSAYE AGAR (SD, SODIUM DIACETATE, P1, 3% PEDIOCIN; P2, 6% PEDIOCIN; SD + P1, SODIUM DIACETATE AND 3% PEDIOCIN; SD + P2, SODIUM DIACETATE AND 6% PEDIOCIN CONCENTRATIONS WERE USED FOR DIPPING) (STANDARD DEVIATION VARIED FROM 0.03 TO 0.54)

surviving *L. monocytogenes* populations of ca. 3.71 log₁₀ cfu/g after 28 d compared to 3% ALTA 2341 alone after 28 d (3.70 log₁₀ cfu/g) of storage. Aerobic bacterial populations in frankfurters treated with antimicrobials and HDP decreased during the storage period (Fig. 3B). In general, populations recovered on TSAYE were greater than those on MOX agar. No antimicrobial treatment significantly reduced aerobic populations in stored frankfurters. Marginal aerobic population reduction was observed during storage of antimicrobials- and HDP-treated frankfurters, similar to *L. monocytogenes* reduction during storage.

DISCUSSION

Our results of MICs of 0.4% sodium diacetate for *L. monocytogenes* are in agreement with Uhart *et al.* (2004), who found MICs of 0.4 to 0.5% among the 4 *L. monocytogenes* strains tested. MIC of pediocin against *L. monocytogenes* has been studied (Jagannath *et al.* 2001; Uhart *et al.* 2004; Bari *et al.* 2005). Szabo and Cahill (1998) observed MICs of ALTA 2341 varying from 160 to 1,200 AU among 21 *L. monocytogenes* isolates in TSB with 0.1% agar. The MIC of pediocin for *L. monocytogenes* Scott A strain was 800 AU/mL in BHI broth (Jagannath *et al.* 2001). The MIC of pediocin for *L. monocytogenes* varied from 1,200 AU for 108 M strain to 30,000 AU for LM 101 M (Uhart

et al. 2004). As the large-scale production of pediocin using *P. acidolactici* was less successful, Chen *et al.* (2002) used ALTA 2341, a commercially available pediocin in their study. Results from various studies suggested that pediocin obtained from different sources had a varied antilisterial effect; depending on *L. monocytogenes* strains and suspending medium used in the determination. The marginal inhibitory activity of antimicrobials used in our study was independent of pH changes as the frankfurter pH remained at approximately 5.9 throughout the 28-d storage period. The slight increase in frankfurter pH during storage could be attributed to the buffering capacity of the meat components (Van Netten *et al.* 1994). The pH increase during storage has been reported with other RTE meat products (Mbandi and Shelef 2001, 2002; Stekelenburg and Kant-Muermans 2001). HDP treatment did not alter the pH of meat samples.

Antilisterial Effect of Antimicrobials

Pediocin has been studied to control spoilage and pathogenic bacterial growth in foods. Marginal *L. monocytogenes* reduction has been noted with the use of pediocin in RTE meats. Pediocin preparation was applied on cooked sausage to determine its antilisterial activity during storage (Mattila *et al.* 2003). They reported ca. 0.5 log₁₀ cfu/g reductions in *L. monocytogenes* following 6 and 21 d of storage at 6C. Dipping treatment of frankfurters in 6,000 AU pediocin resulted in less than 1 log₁₀ cfu/g *L. monocytogenes* reductions after 2 and 3 weeks of storage (Uhart *et al.* 2004). However, when pediocin, SL and sodium diacetate were combined in their study, the reductions ranged from 1 to 1.5 and from 1.5 to 2.5 log units after 2 and 3 weeks of storage, respectively. Nieto-Lozano *et al.* (2006) found that surface spraying of pork ham with 1,000 or 5,000 AU/mL pediocin reduced *L. monocytogenes* populations by 2.5 and 3.5 log₁₀ cfu/g, respectively, following storage of 21 d at 4C. In our study, marginal *L. monocytogenes* reduction with pediocin could be attributed to the inclusion of *L. monocytogenes* strains H7776 and G5854, which were able to grow at 6% ALTA 2341 in MIC study. Our results are in agreement with Samelis *et al.* (2001), who reported that 5% sodium diacetate dipping treatment of pork bologna did not significantly reduce *L. monocytogenes* during storage. The presence of sodium diacetate in frankfurter formulations might have contributed to *L. monocytogenes* reduction in control samples.

Effect of HDP on *L. monocytogenes*

HDP treatment has been evaluated as a nonthermal treatment to inactivate bacterial populations in meats. Based on the limited studies, the bactericidal role of HDP is not clear. Furthermore, the shock waves generated during HDP

treatments are in the range of 70 to 100 MPa for fractions of milliseconds (Williams-Campbell and Solomon 2002). Researchers have shown a significant reduction in the total aerobic populations in ground beef and stew pieces (Williams-Campbell and Solomon 2000, 2002) and in intact beef muscle (Schilling *et al.* 2003). In the present study, HDP treatment alone reduced *L. monocytogenes* populations by ca. 1 log₁₀ cfu/g, which is in agreement with our earlier study with nisin and HDP treatment (Patel *et al.* 2007). Other treatments have been used in combination with antimicrobial treatments to enhance bactericidal effects. Spraying of 6,000 AU ALTA 2341 on 1- or 5-link frankfurters followed by postpackaging irradiation at 1.2 kGy or more resulted in 50% reduction of *L. monocytogenes* (Chen *et al.* 2004). High-pressure processing (HPP) has been studied as a postlethal treatment in RTE meats (Lucore *et al.* 2000; Hayman *et al.* 2004). Garriga *et al.* (2002) found significant *L. monocytogenes* reduction in model meat system using HPP and pediocin. As with HPP, HDP treatment may be combined with other antimicrobials to enhance its bactericidal effects.

HPP resulted in the lower recovery of pathogenic bacteria on selective media because of sublethal injury (Kalchayanand *et al.* 1994). These injured cells are sensitive to bacteriocins and subsequently die in the presence of antimicrobials (Garriga *et al.* 2002). In our study, relatively higher populations of *L. monocytogenes* were detected on nonselective TSAYE medium compared to the population detected on selective MOX media. The pressure fronts generated during HDP treatment could have sensitized injured bacteria to bacteriocin in a manner similar to HPP (Masschalck *et al.* 2001).

From the results of our study, it is clear that *L. monocytogenes* survived at 4C for 4 weeks in the presence of organic acids and pediocin, necessitating a storage temperature of less than 4C to prevent *Listeria* survival and/or growth. Pediocin and sodium diacetate treatments used in the study are not efficient enough to kill *L. monocytogenes* and therefore other antimicrobials may be used to prevent postprocessing contamination of RTE meat products with *L. monocytogenes*. Combination of pediocin with sodium diacetate does not enhance the antilisterial effect of pediocin. There is a potential for cells to develop resistance to bacteriocins during prolonged exposure. Such a problem could be avoided with other antimicrobial interventions such as high-pressure treatment. Other antimicrobials should be studied in combination with the postprocessing treatment like HDP to determine its efficacy in reducing *L. monocytogenes* populations in RTE meats.

REFERENCES

- BARI, M.L., UKUKU, D.O., KAWASAKI, T., INATSU, Y., ISSHIKI, K. and KAWAMOTO, S. 2005. Combined efficacy of nisin and pediocin with

- sodium lactate, citric acid, phytic acid, and potassium sorbate and EDTA on reducing *Listeria monocytogenes* population of inoculated fresh-cut produce. *J. Food Prot.* 68(7), 1381–1387.
- BARMPALIA, I.M., GEORNARAS, I., BELK, K.E., SCANGA, J.A., KENDALL, P.A., SMITH, G.C. and SOFOS, J.N. 2004. Control of *Listeria monocytogenes* on frankfurters with antimicrobials in the formulation and by dipping in organic acid solutions. *J. Food Prot.* 67(11), 2456–2464.
- CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC). 1998. Multistate outbreak of listeriosis. *MMWR Morb. Mortal. Wkly. Rep.* 47(50), 1085–1086.
- CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC). 2002. Public health dispatch: Outbreak of listeriosis – Northeastern United States, 2002. *MMWR Morb. Mortal. Wkly. Rep.* 51(42), 950–951.
- CHEN, C.M., SEBRANEK, J.G., DICKSON, J.S. and MENDONCA, A.F. 2004. Combining pediocin with postpackaging irradiation for control of *Listeria monocytogenes* on frankfurters. *J. Food Prot.* 67(9), 1866–1875.
- CHEN, C.M., SEBRANEK, J.G., DICKSON, J.S. and MENDONCA, A.F. 2002. Use of pediocin with other barriers for control of *Listeria monocytogenes* on ready-to-eat (RTE) processed meats. Project report submitted to American Meat Institute Foundation, April, 2002. <http://www.amif.org/ht/a/GetDocumentAction/i/7410> (accessed December 1, 2006).
- GARRIGA, M., AYMERICH, M.T., COSTA, S., MONFORT, J.M. and HUGAS, M. 2002. Bactericidal synergism through bactericins and high pressure in a meat model system during storage. *Food Microbiol.* 19, 509–518.
- GILL, A.O. and HOLLEY, R.A. 2000. Inhibition of bacterial growth on ham and bologna by lysozyme, nisin, and EDTA. *Food Res. Int.* 33, 83–90.
- HAYMAN, M.M., BAXTER, I., O'RIORDAN, P.J. and STEWART, C.M. 2004. Effects of high-pressure processing on the safety, quality, and shelf-life of ready-to-eat meats. *J. Food Prot.* 67, 1709–1718.
- JAGANNATH, A., RAMESH, A., RAMESH, V.N., CHANDRASHEKAR, A. and VARADARAJ, M.C. 2001. Predictive model for the behavior of *Listeria monocytogenes* Scott A in shrikhand, prepared with a biopreservative, pediocin K7. *Food Microbiol.* 18, 335–343.
- JAY, J.M. 1996. Foodborne listeriosis. In *Modern Food Microbiology* (J. Jay, ed.) pp. 478–506, Chapman and Hall, New York, NY.
- KALCHAYANAND, N., SIKES, A., DUNNE, C.P. and RAY, B. 1994. Hydrostatic pressure and electroporation have increased bactericidal efficiency in combination with bacteriocins. *Appl. Environ. Microbiol.* 60(11), 1–4.

- LU, Z., SEBRANEK, J.G., DICKSON, J.S., MENDONCA, A.F. and BAILEY, T.B. 2005. Inhibitory effects of organic acid salts for control of *Listeria monocytogenes* on frankfurters. *J. Food Prot.* 68, 499–506.
- LUCHANSKY, J.B. and CALL, J.E. 2004. Evaluation of nisin-coated cellulose casings for the control of *Listeria monocytogenes* inoculated onto the surface of commercially prepared frankfurters. *J. Food Prot.* 67, 1017–1021.
- LUCORE, L.A., SHELLHAMMER, T.H. and YOUSEF, A.E. 2000. Inactivation of *Listeria monocytogenes* Scott A on artificially contaminated frankfurters by high-pressure processing. *J. Food Prot.* 63, 662–664.
- MASSCHALCK, B., HOUDT, R.V. and MICHIELS, C.W. 2001. High pressure increases bactericidal activity and spectrum of lactoferrin, lactoferricin and nisin. *Int. J. Food Microbiol.* 64, 325–332.
- MATTILA, K., SARIS, P. and TYOPPONEN, S. 2003. Survival of *Listeria monocytogenes* on sliced cooked sausage after treatment with pediocin AcH. *Int. J. Food Microbiol.* 89, 281–286.
- MBANDI, E. and SHELEF, L.A. 2001. Enhanced inhibition of *Listeria monocytogenes* and *Salmonella* Enteritidis in meat by combination of sodium lactate and acetate. *J. Food Prot.* 64, 640–644.
- MBANDI, E. and SHELEF, L.A. 2002. Enhanced effects of combinations of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. *Int. J. Food Microbiol.* 76, 191–198.
- MEAD, P.S., SLUTSKER, L., DIETZ, V., MCCAIG, L.F., BRESEE, J.S., SHAPIRO, C., GRIFFIN, P.M. and TAUXE, R.V. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5, 607–625.
- NIETO-LOZANO, J.C., REGUERA-USEROS, J.I., PELAEZ-MARTINEX, M.C. and TORRE, A.H. 2006. Effect of bacteriocin produced by *Pediococcus acidolactici* against *Listeria monocytogenes* and *Clostridium perfringens* on Spanish raw meat. *Meat Sci.* 72, 57–61.
- PATEL, J.R., BHAGWAT, A.A., SANGLAY, G.C. and SOLOMON, M.B. 2006. Rapid detection of *Salmonella* from hydrodynamic pressure-treated poultry using molecular beacon real-time PCR. *Food Microbiol.* 23, 39–46.
- PATEL, J.R., SANGLAY, G.C., SHARMA, M. and SOLOMON, M.B. 2007. Combining antimicrobials with hydrodynamic pressure processing for control of *Listeria monocytogenes* on frankfurters. *J. Muscle Foods* 18, 1–18.
- PATEL, J.R. and SOLOMON, M.B. 2005. Attachment of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* to beef and inactivation using hydrodynamic pressure processing. *Proc. SPIE-Int. Soc. Opt. Eng.* 5996, E1–E6.

- PEACHEY, B.M., PURCHAS, R.W. and DUIZER, L.M. 2002. Relationships between sensory and objective measurements of meat tenderness of beef *M. longissimus thoracis* from bulls and steers. *Meat Sci.* 60, 211–218.
- SAMELIS, J. and METAXOPOULOS, J. 1999. Incidence and principal sources of *Listeria* spp. and *Listeria monocytogenes* contamination in processed meats and a meat processing plant. *Food Microbiol.* 16, 465–477.
- SAMELIS, J., SOFOS, J.N., KAIN, M.L., SCANGA, J.A., BELK, K.E. and SMITH, G.C. 2001. Organic acids and their salts as dipping solutions to control *Listeria monocytogenes* inoculated following processing of sliced pork bologna stored at 4C in vacuum packages. *J. Food Prot.* 64, 1722–1729.
- SCHILLING, M.W., MARRIOTT, N.G., WANG, H. and SOLOMON, M.B. 2003. Characteristics of USDA utility cow beef subjected to blade tenderization and hydrodynamic shockwaves. *J. Muscle Foods* 14, 131–142.
- SOLOMON, M.B., LONG, J.B. and EASTRIDGE, J.S. 1997. The hydrodyne: A new process to improve beef tenderness. *J. Anim. Sci.* 75, 1534–1537.
- STEKELENBURG, F.K. and KANT-MUERMANS, M.L.T. 2001. Effects of sodium lactate and other additives in a cooked ham product on sensory quality and development of a strain *Lactobacillus curvatus* and *Listeria monocytogenes*. *Int. J. Food Microbiol.* 66, 197–203.
- SZABO, E.A. and CAHILL, M.E. 1998. The combined effects of modified atmosphere, temperature, nisin and ALTA™ 2341 on the growth of *Listeria monocytogenes*. *Int. J. Food Microbiol.* 43, 21–31.
- UHART, M., RAVISHANKAR, S. and MAKES, N. 2004. Control of *Listeria monocytogenes* with combined antimicrobials on beef franks stored at 4C. *J. Food Prot.* 67, 2296–2301.
- UNITED STATES DEPARTMENT OF AGRICULTURE, FOOD SAFETY AND INSPECTION SERVICE. 2003. Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products. *Fed. Regist.* 68, 34207–34254.
- VAN NETTEN, P., HUIS, J.H.J. and MOSSEL, D.A.A. 1994. The immediate bactericidal effect of lactic acid on meat-borne pathogens. *J. Appl. Bacteriol.* 77, 490–496.
- WILLIAMS-CAMPBELL, A.M. and SOLOMON, M.B. 2000. New non-thermal postharvest technology to improve food safety: Hydrodynamic pressure processing. *Proc. SPIE-Int. Soc. Opt. Eng.* 4206, 167–173.
- WILLIAMS-CAMPBELL, A.M. and SOLOMON, M.B. 2002. Reduction of spoilage microorganisms in fresh beef using hydrodynamic pressure processing. *J. Food Prot.* 65, 571–574.